EMBRYOLOGY.[1]

Experimental Embryology.—Under this title may be included two most interesting recent contributions to our knowledge of the significance of the cleavage phenomena in animal eggs. Both deal with the cleavage phenomena of eggs influenced by experimental interference; the one in the case of a Vertebrate, the other in an Echinoderm.

Prof. E. B. Wilson,[2] of Columbia College, finds that the cleavage cells of an Amphioxus egg when shaken apart are yet capable of developing into gastrulas or even into complete larvæ of perfectly normal structure. Yet the *size* of the gastrulæ or larvæ is determined by the size of the cleaving cell; thus if one of the first two cells is taken the resulting gastrula is half the normal size, if one of the first four is taken, the gastrula is one-fourth the normal bulk. Gastrulas of one-eighth the normal size occurred but were not products of isolated cells in the eight-cell stage; in fact, the experiments go to show that one of these first eight cells is incapable of forming a gastrula. There is thus an early limit set upon the potentialities of the cleavage cells. This, moreover, seems to be a qualitative and not a quantitative limit. The homogeneous, undifferentiated character of the cells is lost at the eight-celled stage.

When the cells are incompletely separated, double, triple, and even quadruple embryos are formed.

Comparing the development of the isolated cells of Amphioxus with what is known of similar phenomena in other Vertebrates as known from the work of Roux upon the frog, or with the facts established for Echinoderms by Dreisch and others, we find an important and fundamental difference in the time at which individuation and symmetry become apparent. While in the Echinoderm the partial development proceeds at first as if all the cells were present, and only later what may, improperly, be called a process of *regeneration*, forms that which was lacking to the completion of a normal symmetrical embryo, in Amphioxus, as the author shows by diagrams giving more accurate observations than had hitherto been made upon the exact mode of cleaving,—"the isolated

[1]This department is edited by Dr. E. A. Andrews, Johns Hopkins University.

[2]On Multiple and Partial Development in Amphioxus. Anat. Anz., 1892, Nos. 23–24.

blastomere develops as a unit, not as a half-unit; and the cells to which it gives rise cannot be individually identified with those of a normal embryo-half. The development is transformed from the beginning."

The second paper, by Hans Dreisch, of Zürich,[3] contains many important facts not presented in the preliminary article noticed in the December NATURALIST.

When the eggs of certain sea-urchins are exposed to high temperatures an acceleration in the rapidity of cleavage processes is not the only result—but at a temperature of 31° C. certain marked changes both in the size and position of cells, the entire absence of micromeres and other such fundamental effects are produced. Nevertheless eggs that cleave in these abnormal ways may develop into perfectly normal larval forms, plutei. Long continued exposure to such temperatures acts like the mechanical force applied in shaking eggs in a test-tube and results in the separation of cleaving eggs into two, rarely more than two portions, each of which may then develop by itself. As some of these portions form normal larvæ, high temperature may be one of the factors concerned in the formation of multiple embryos.

Returning to the cleavage of cells separated by shaking echinoderm eggs, a series of figures are given to illustrate the fact that the isolated cells continue to cleave, for a time, as if still in combination with their fellow cells. Thus three cells taken from a four-celled stage do not at once fill up the gap left by the removal of the fourth and this fourth cell divides by itself just as it would have done if remaining in contact with the three others. It is only at a later stage that the products of isolated cells arrange themselves so as to form a complete, symmetrical individual.

A portion of the paper is taken up with certain abnormal methods of cleavage, beginning with the simultaneous formation of four instead of two cells, at the first cleavage. In these cases the cleavage continues to be *double*, a sixteen-cell stage having two sets of each eight cells comparable to the entire eight-cell stage of a normal egg. No larvæ could be reared from such eggs, yet special interest attaches to them upon the assumption that they owe their dual nature to a double fertilization. The only evidence that these eggs were fertilized by two sperms is the assumption by Fol and others that double fertilization causes the formation of four in place of the normal two first cleavage cells.

A most important addition to the methods of experimentation has resulted in unexpected results. It consists in subjecting echinoderm

[3]Entwicklungsmechanische Studien. Zeit. f. Wiss. Zool., 55, 1892.

eggs to mechanical pressure by the very simple expedient of allowing a cover-glass to rest upon them with more or less force as controlled by a hair inserted under the glass.

Eggs flattened out to several times their original diameter do not lose their power to develop, even when the egg membrane is ruptured. If the eggs are fertilized and undergo cleavage under pressure the resulting cells are not arranged in the form of a sphere but form a flat disk. This arrangement of the cells results from the arrangement of the nuclear spindles. While in a normal egg the spindles of the first four cells would stand vertically, as it were, and produce a new set of cells by a horizontal cleavage, under pressure the spindles are found all lying at right angles to the compressing force; that is, parallel to the cover glass or horizontally, and hence give rise to cells all lying in one plane. When the pressure is removed the cells remain at first in a flat disk, though each cell becomes rounded. Subsequent divisions of these cells give rise to a spherical mass capable of forming a complete normal embryo.

With the aid of figures the author makes it clear that in such cases material which would normally have taken part in the formation of only one pole or side of the embryo must now contribute to opposite poles or sides. In the sea-urchin there is thus no early specialization or differentiation of the material found in the cleavage cells; the mixing up of the blastomeres would not prevent the formation of a complete normal animal: the author concludes they could be mixed in all possible ways without destroying the normal symmetry of the adult.

In the philosophical discussion that takes up the final portion of the paper, the author considers the methods of morphological research and upholds the experimental method, which tends to promise the reduction of Biology to a scientific basis sooner than the descriptive, historical or mechanical lines of inquiry possibly can.

Studies in Insect Embryology.—Dr. H. Henking[4] having previously published extensive researches upon the formation, maturation and union of the sexual cells in *Pieris brassica* and *Pyrrhocoris apterus* concludes this comparative study with additional facts upon the eggs of the latter insect as well as those of *Agelastica alni*, *Donacia lampyris*, *Tenebrio*, *Lasius*, *Rhodites*, *Bombyx*, *Musca*, and other representatives of nearly all the chief groups of insects and then summarizes the whole with the aid of most interesting comparisons and suggestions.

[4] Zeit. f. Wiss. Zool., 54, 1892; 51, 1891; 49, 1890.

Along with the observation of normal processes, attempts were also made in some cases to influence these processes by increasing or diminishing the atmospheric pressure, by placing the egg in horizontal and vertical positions, by surrounding them with CO_2 and with O; but the results are not definite enough to afford more than suggestions for future work.

Regarding the maturation of the egg the author holds views that depend upon the interpretation of peculiar appearances seen in insect eggs, where, as is well-known, the technique is a most difficult factor.

The first polar body receives one-half of the chromosomes, one-half the normal number of the ordinary cell, and also the first *thelyid.* This *thelyid* or "acromatic polar body," is a rather vague mass of acromatic substance including the connecting filaments and adjacent substances between the two separating sets of chromosomes.

The second polar body receives the same number of chromosomes as the first, these being formed by a division of each, so that a half of each remains to form the egg nucleus; in some cases there is a second *thelyid,* but this remains inside the ovum.

The two polar bodies may separate completely from the egg, but more often they remain either in protoplasmic processes of its surface or entirely beneath its surface; the first polar body may divide into two.

Even when the polar bodies are extruded they may be later taken into the egg again. Their nuclei may undergo changes similar to that of the egg nucleus, either retaining the separate chromosomes or forming a chromatic network. The first and second or the product of the division of the first and second may fuse with one another inside the egg, much as the sexual pronuclei do.

Meanwhile numerous sperms may enter an egg; polyspermy in insects has no apparent injurious results. The sperm passes into the yolk in a bent attitude, with the union of head and tail preceding. A clear area about the sperm, the *arrhenoid,* seems to give rise to radiating striæ in the protoplasm. This peculiar body is regarded as formed from the acromatic connecting filaments given to the sperm at the second division of its mother cell; is thus comparable, in a way, to the *thelyid.* Increase in size and change in the sperms take place simultaneously in all that penetrate the yolk; only one meets and unites with the female pronucleus.

The number of chromosomes in the nuclei of ordinary somatic cells and in the cleaving egg is 28, 24, 20, 18–20, 24–30, in five different insects studied; the number in the first and also in second polar bodies in these same species, 14, 12, 10, 9, about 12. As far as studied the

spermatocytes have also this same half-number after their first and second divisions; with some interesting exceptions the sperm and the polar body and the female pronucleus have the same number, in any species, and this is half the number of other cells of that species.

With the aid of a diagram the author emphasizes the close parallel between the formation of sperms and polar bodies. Starting from a mother cell with, say 24 chromosomes, a *reducing* division forms two sperm mother cells, or in the female an egg and a polar body, in each of which are only 12 chromosomes, and these are half of the original 24, unchanged or undivided individually. A second division of each cell results in the formation of four sperm mother cells or in the female of three polar bodies, and the remaining egg, in each of which are 12 chromosomes. These, however, are formed by an *equal* division, that is, from the previous 12, 24 arise by a division of each—not by the separation of the whole set into two sets as in division with *reduction*. Later the 12 chromosomes of a sperm may be added to the 12 of the egg nucleus to make the normal 24 of the cleavage nucleus, whence, by equal division, all subsequent cells have 24 also. Likewise, in the insects, one polar body may unite its 12 with the 12 of another polar body.

Utilizing the recent researches of Guignard,[5] the author draws a most interesting comparison between the processes taking place in insects and in plants. Without the aid of diagrams this can scarcely be rendered intelligible; the most novel part, however, is an attempt to explain the polar bodies of animal eggs as, in a sense, "larval organs," somewhat comparable to rudimentary structures within the embryo sac of a flowering plant.

The fact that in insects the polar bodies may remain within the egg and that they, or their descendants, may unite with one another, seems to form a parallel to what takes place in the flowering plant. As is well-known, the embryo-sac cell forms two sets of each 4 nuclei within its substance, 4 lower antipodal cells and 4 upper cells, of which one is the ovum.

The formation of these is directly comparable to the formation of polar bodies in the animal—supposing a second series of divisions to supervene and convert the 4, ovum and three polar bodies, into eight. In the embryo-sac a remarkable fusion takes place between one of the antipodal nuclei and the sister cell of the ovum; this union forms the endosperm. Compared with the insect, this union is like the fusion of polar bodies, only in a later generation. In the insect nothing appar-

[5]See this Journal, May, 1892.

ently results from the fusion of polar bodies; they are rudimentary larval organs.

The fact that we here compare union of later generations in the plant with that of earlier ones in the insect or animal is perhaps made less objectionable by considering the state of things in the infusoria, where, as Maupas has shown, the final fusion takes place between nuclei resulting from more numerous divisions than are employed in the formation of sperm and female pronucleus.

The observations of Henking upon the number and division of the chromosomes in insects conflict in an important particular with the facts necessary to support Weismann's theoretical explanation of the use of polar bodies. In his "Amphimixis" Weismann holds that *reduction* takes place in the formation of both polar bodies, and that there was previously a doubling in the number of chromosomes or *idanten* to allow of greater complexity of combination of the *iden* or hypothetical hereditary units found in the chromosomes. Hinking denies that the second polar body is formed by *reduction;* it is formed by an *equal* division. Moreover, there is no previous doubling of chromosomes; the apparent doubling is only a more or less pronounced division into parts still remaining subservient to the whole, not acquiring individuality.

Weismann's views are moreover limited in that they depend upon the occurrence of two polar bodies, two divisions in the formation of sexual cells; whereas in plants and in infusoria, nuclei that need to copulate result from more numerous divisions.

Parthenogenesis again, as seen in hymenoptera and lepidoptera, may occur where two polar bodies with a smaller number of chromosomes are present.

Though recognizing the strength of the evidence that heredity is closely associated with the chromosomes, the author thinks they are not unchangeable, and that at times they may be more intimately combined with the other parts of the nucleus. If the centrosomes are to be regarded in heredity as of like value with the chromosomes then we must seek in them a process of reduction before copulation.

All present theories of fertilization and of inheritance have only a provisional character.